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Effect of a three-herb formulation on chronic inflammation induced by Freund's Complete Adjuvant in Wistar rats

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Abstract

New innovative therapies are urgently needed to combat the high morbidity associated with sickle cell crises. Given the many side effects associated with conventional anti-inflammatories, the use of herbal medicines would be beneficial for patients. In an effort to help manage chronic inflammation in sickle cell disease, a three-herb recipe was used to test the anti-inflammatory activity elicited by Freund's Complete Adjuvent in Wistar rats. Rats' pulp volume and hematological parameters were measured. After the 20 days of treatment, the extracts from the recipe reduced the edema caused by CFA and the proliferation of the number of leukocytes, the level of fibrinogen and also increased the level of hemoglobin which is the key parameter of anemia. These observed effects are due to the antioxidant power of phenolic compounds and alkaloids, the terpenoids present in the extracts of ZHm.

Keywords: Herb Formulation; Chronic Inflammation; Freund's Complete Adjuvent; Wistar Rats

1. Introduction

Sickle cell disease is due to a mutation in the β-globin gene, resulting in the production of hemoglobin S (HbS). In situations of hypoxia, acidosis, and or a high level of 2-3 DPG, the solubility of hemoglobin S is decreased, and it will polymerize by gelling, resulting in its dehydration. Thus leading to the deformation of normal red blood cells into abnormal red blood cells or sickle cell [1]. This primary pathophysiological alteration has innumerable consequences, including the production of inflammatory molecules and responses that ultimately lead to vaso-occlusion of microvessels, which is one of the main manifestations of sickle cell disease [2]. Among the mediators of inflammation, we can cite : chemokines, which are leukocyte activating proteins with chemotactic properties that attract these cells to the site of inflammation, then cytokines, which are proteins secreted by leukocytes that ensure intercellular communication and adhesion molecules. Inflammation plays a major role in the physiopathology of sickle cell disease [3]. It is the response of living, vascularized tissues to an aggression. Although it is difficult to identify the exact events that trigger the chronic inflammatory state that defines sickle cell disease, the literature lists a number of pathophysiological mechanisms that may contribute to inflammation such as red blood cell alteration, hemolysis, oxidative stress, molecular patterns associated with microbiota-derived pathogens [4] and gut pathogens [5]. Management of inflammation involves active innate biochemical mechanisms and anti-inflammatory molecules that allow inflamed tissues to return to homeostasis [6]. Currently, treatment options for sickle cell disease are limited to hematopoietic stem cell transplantation and Crisper Cas9 gene therapy [7,8], which are a curative option but limited in availability to low-income populations. Chronic transfusion, hydroxyurea therapy and NSAIDs remain the only alternatives for these populations.

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Although the U.S. Food and Drug Administration recently approved L- glutamine for use in sickle cell disease, it remains inaccessible and costly for low-income populations [9]. Thus, to address chronic and non-chronic diseases several plants are used by rural populations, and these plants have scientifically proven their therapeutic effects in inflammation, oxidative stress, infection, anemia, hemolysis. In order to contribute to the management of chronic inflammation in sickle cell disease, a three-herb recipe was used to test the anti-inflammatory activity of Freund's Complete Adjuvent in Wistar rats.

2. Materials and Methods

2.1. Plant Material

Fruits of *Xylopia aethiopica* (Dunal) A. Rich*,* barks *of Zanthoxylum leprieurii* (GUILL), and leaves of *Harungara madagascariensis* (LAM) were collected in January 2018 from the Indenie-Djuablin region of Eastern Côte d'Ivoire. For each batch of a given species, not only were foreign species removed, but also the harvests were cleared of samples that were soiled, rotted, burned by fire, or contaminated by mold or any other pest. Except for the bark, the fruits and leaves were rinsed, shaken dry and cut into small pieces. These plant pieces were then dried in the shade at room temperature (25-30°C) for four weeks. The drying was done in a well-ventilated room. After drying, the plant parts (of each individual plant species) were finely ground using an IKA-Labortechnik electric grinder (Type MFC /Janke & Kunkel). Based on the information collected from the traditional healers, the recipe was compiled. The powders obtained after pulverization of these plants were assembled in the quantities of 33.33 ± 1 g per plant and the new composition obtained was coded ZHm.

2.2. Animal Material

Nulliparous and non-pregnant Wistar rats weighing between 104 and 130 g from the animal house of the Higher Normal School of Abidjan (Côte d'Ivoire) were used. These animals were placed in conditions where the ambient temperature was between 26 and 30°C, humidity between 40% and 60%, and lighting of 12 hours of light and 12 hours of darkness. The rats had free and continuous access to water and food. All procedures and techniques used in this experiment were performed according to the National Institute of Health guidelines for the care and use of laboratory animals.

2.3. Preparation of Extracts

2.3.1. Preparation of the Aqueous Extract

Following the method of Tatiana [10], one hundred grams (100 g) of the plant powder mixture was boiled for 20 min in 2 L of distilled water. The decoctate was cooled to room temperature (25°C) and filtered three times through absorbent cotton and once through Whatman 3 mm filter paper. The filtrate was then dried at 50°C using a Venticell® type oven. The powder obtained was the total aqueous extract coded DZHm.

2.3.2. Preparation of the Hydroethanol Extract

The hydroethanol extract was prepared according to the method of Tatiana [10]. One hundred grams (100 g) of the plant powder mixture were dissolved in one liter of the hydroalcoholic solvent comprising 70% ethanol and 30% distilled water. The mixture was then homogenized using a Severin ® brand blender. The homogenate obtained was wrung out in a cloth square and then filtered three times on hydrophilic cotton and once on whatman paper (3 mm). The filtrate was evaporated at 45°C using a Venticell® type oven for 24 hours. The dry powder obtained was the hydroethanol extract coded EZHm.

2.4. Freund's Complete Adjuvant (FCA) induced chronic inflammation

Chronic inflammation was induced according to the method of Pearson [11]. It was induced in rats aged six to eight weeks by the injection of 40 µl of Freund's complete adjuvant solution under the plantar fascia of the right hind leg of the rats. The diameter of the injected leg was measured by digital caliper at day zero (before injection) and at days 1; 4; 8; 12; 16 and 20 (after injection) of Freund's complete adjuvant solution. Treatment began the day after injection of Freund's adjuvant and continued for twenty days. Eight groups of six rats each (three males and three females) received the following oral treatments once daily:

- A healthy control group receiving distilled water.
- A control group receiving distilled water.
- The second groups was treated with diclofenac 5 mg/kg body weight (bw).

- The third, fourth and fifth groups were treated with the ZHm decocted (DZHm) at 200, 400 and 800 mg/kg/bw respectively.
- The sixth, seventh and eighth groups treated with the ZHm hydroethanol extract (EZHm) at 200, 400 and 800 mg/kg/bw respectively.

Rat weights were recorded before induction of inflammation, and after induction at four-day intervals for fourteen days. At the end of the experimental period, the animals were anaesthetized with ether after 18 hours of fasting. Blood was collected in EDTA-containing tubes and citrated tubes for the respective determinations of haematological parameters and fibrinogen level.

The importance of arthritis is assessed by determining the percentage increase in leg diameter (% AUG) of the animal.

$$\%AUG = [(Dtn-Dt0) /Dt0] *100$$

Dtn: Diameter of the leg at time (tn)
Dt0: Diameter of the leg at time (t0)

The anti-inflammatory activity was evaluated by calculating the percentage inhibition of edema (% INH).

$$\%INH = [(\%AUGcontrol-\%AUGtreated) / \%AUGcontrol] *100$$

2.5. Statistical Analysis

All results were expressed as mean ± the error on the mean (ESM). Statistical analysis and graphs were performed using Graphpad prism version 9 software. Comparison between three or more groups was evaluated using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The level of significance was set at $P < 0.05$.

3. Results

3.1. Yield

The yield values were calculated relative to the initial mass of ZHm powder for one trial. The extraction yields of the plant were 13.13% for the ethanolic extract 70% (EZHm) and 10 % for the aqueous extract (DZHm).

3.2. Effect of ZHm extracts on chronic inflammation

3.2.1. Effect of extracts on body mass

During the 20 days of treatment, the weight of the rats gradually increased as a function of time. Twenty-four (24) hours after the injection of Complete Freund's Adjuvant (CFA), the weight of the rats of all the experimental batches experienced a non-significant decrease. Body weight gain in all animals treated with ZHm extracts was substantially the same and there was no significant difference ($p > 0.05$) between treated rats and diseased controls (Fig1-A and Fig2-A).

3.2.2. Effect of extracts on paw edema

In all batches that underwent the injection of Complete Freund's Adjuvant (CFA), signs of inflammation appeared from the first day of injection. Rat paw volume increased in all experimental groups with diameters ranging from 4.57 to 4.98 mm. The edemas also increased after the various treatments. These signs reached their maximum level on the 4th day with the thicknesses of 6.1±0.29 mm, 5.36±0.15mm, 5.55±0.15 mm, 5.68±0.23 mm, 5 .70±0.08 mm and 5.61±0.14 mm respectively for the edematous control batches, Diclofenac, 200, 400 and 800 mg/kg Bw. The percentage increase in edema corresponds to 125.7%, 93.92%, 100.4%, 107.9%, 95.3% and 93.58% respectively (Fig1-B).

The treated batches showed moderate signs of inflammation compared to the arthritis controls. The results show a reduction in the volume of the edematous paw of rats treated with the extract and the reference molecules from the 4th day of treatment until the end of the experiment with Diclofenac and DZHm. The percentages of inhibition of the rats treated with Diclofenac and DZHm at the doses of 200, 400 and 800 mg/kg bw were respectively 38.17%, 21.09%, 31.73% and 28.41%. This inhibition was progressive until the 20th day to reach the respective inhibition rates of 54.87%, 47.07%, 55.81% and 56.88%. In general, all the substances tested inhibited edema in rats in a dose-dependent manner. Doses of 400 and 800 mg/kg/bw had anti-inflammatory activity similar to that of Diclofenac (Fig1-C).

As for the rats treated with the hydroethanolic extract (EZHm), the analysis of the results shows that the extract gradually inhibited the edemas in a dose-dependent manner. On the 4th day of treatment, the inhibition rate was 10.86%, 40.39% and 36.96%; then on the 20th day, it was 17.75%, 49.58% and 52.47% at doses of 200, 400 and 800 mg/kg bw respectively.

Analysis of the results reveals that the maximum activity of the ZHm extracts was observed on the 12th day of treatment. At the end of the treatment, the dose of 800 mg/kg bw of EZHm (52.47%) and DZHm (56.88%) generated an activity similar to that of Diclofenac (54.87%) (Fig 2-A, B and C).

3.3. Effect of extracts on hematological parameters

The analysis of the results shows a modification of the hematological parameters of the control rats, the untreated rats and the rats treated for 20 days of experience. Chronic inflammation achieved with CFA caused an increase in white blood cells (WBC), blood platelets and fibrinogen, followed by a decrease in red blood cells (RBC), hemoglobin (Hb) and hematocrit (Hte) in CFA-treated rats compared to healthy control rats (Table 1 and 2).

A non-significant ($p > 0.05$) increase in white blood cells was observed between healthy controls (15.58 x103 /µL) and untreated rats (21.12 x103 /µL). No significant difference ($p > 0.05$) was observed between the blood platelets of healthy control rats and those of untreated rats. A significant increase ($p<0.001$) in the level of fibrinogen was noted in the untreated rats (3.5 g/L) and in the control rats (2 g/L). Although the number of red blood cells, hemoglobin level and hematocrit level decreased, no significant difference ($p > 0.05$) was observed between healthy control rats and untreated rats.

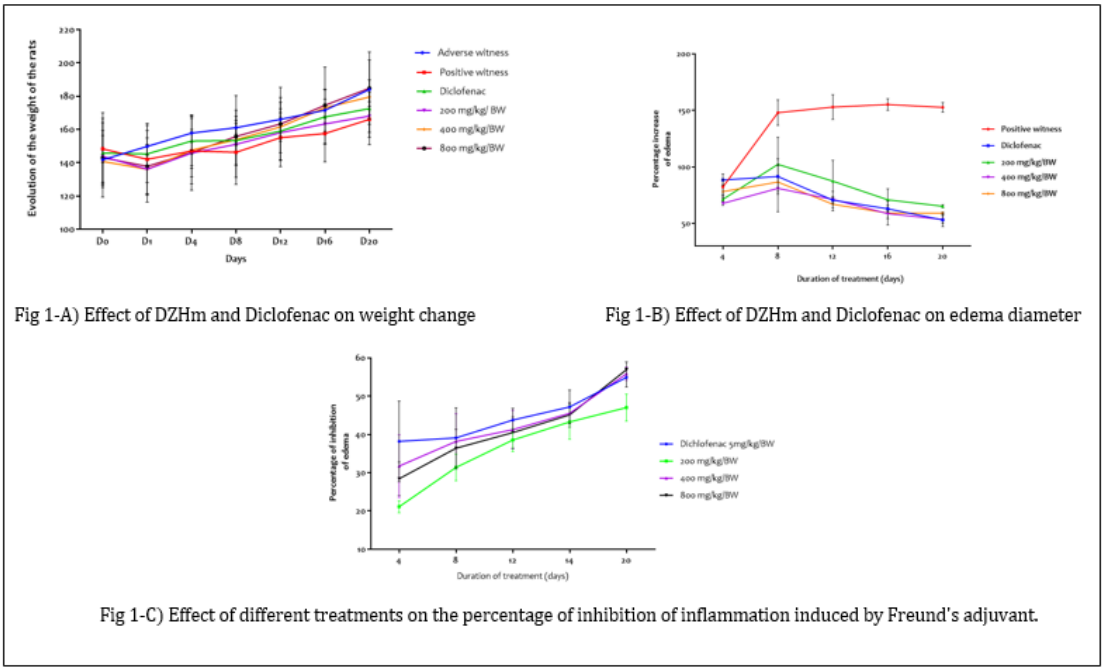

Fig 1-A) Effect of DZHm and Diclofenac on weight change

Fig 1-B) Effect of DZHm and Diclofenac on edema diameter

Fig 1-C) Effect of different treatments on the percentage of inhibition of inflammation induced by Freund's adjuvant.

Figure 1 Effect of decocted extract (DZHm) on inflammation caused by CFA

Daily administration of DZHm to rats at doses of 200, 400 and 800 mg/kg bw for 20 days caused a decrease in the number of white blood cells and the level of fibrinogen in them. This decrease in the number of white blood cells was respectively 12.45 x103 µL; 14.01 x103µL; 12.12 x103 µL and 12.76 x103 µL in the rats treated with Diclofenac as well as those of the rats treated with the doses of 200, 400 and 800 of DZHm, while the number of white blood cells of the control rats was 21, 12x103µL. As for the fibrinogen level, it was 3.5 g/L and 2.52 g/L for the untreated rats (CFA) and the rats treated with Diclofenac then respectively 2.3 g/L; 1.65 g/L and 2 g/L for those treated with 200; 400 and 800 mg /kg.bw of DZHm.

The administration of DZHm induced a significant increase ($P < 0.05$) in the hemoglobin level and the number of red blood cells of the treated rats. The hemoglobin level of the untreated rats (CFA) was 12.03 g/dL and that of the treated rats was 12.63 g/dL for Diclofenac, 12.85 g/dL; 13.88 and 14.13 g/dL for rats treated with DZHm at doses of 200, 400 and 800 mg/kg bw respectively. The administration of DZHm also generated a significant increase ($P < 0.05$); ($P < 0.01$) of the number of red blood cells of the rats treated with the extract at the respective doses of 400 and 800 mg/kg bw compared to the untreated control rats. However, no significant difference ($p > 0.05$) was observed between the number of red blood cells of the untreated rats and that of the rats treated with Diclofenac and the aqueous extract at 200 mg/kg bw.

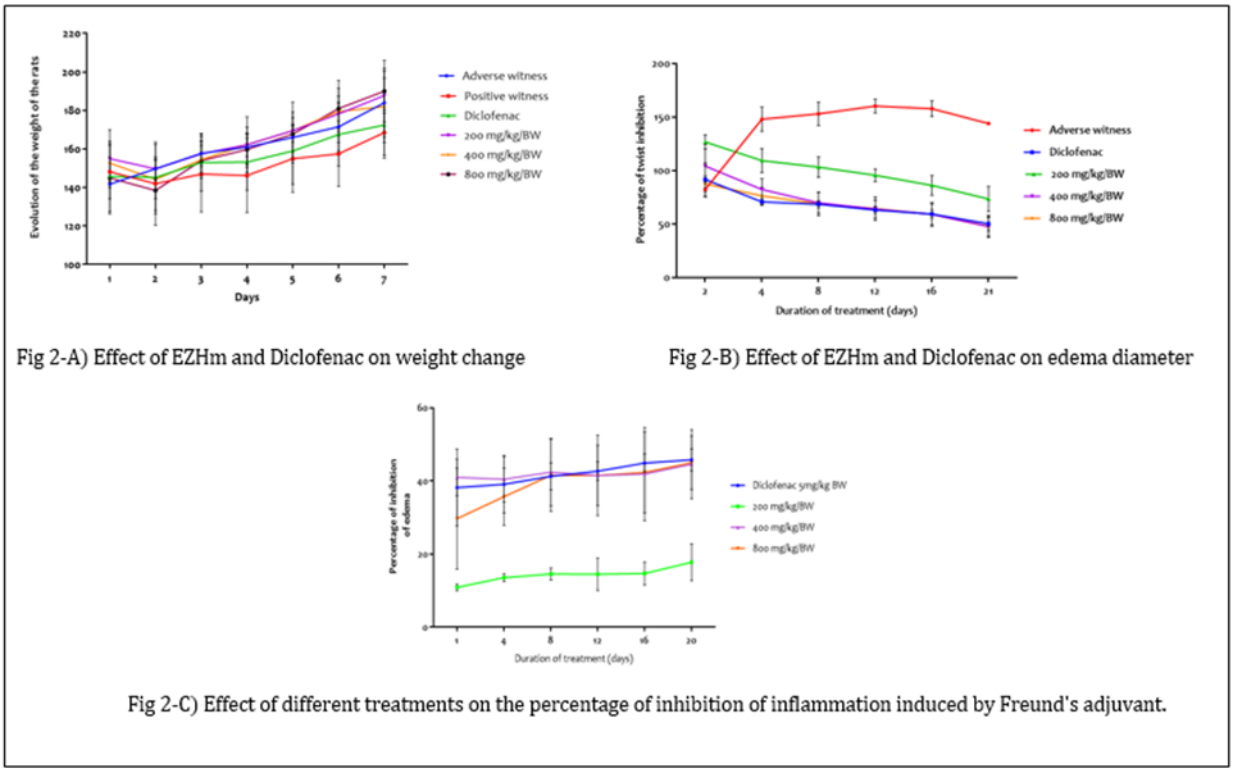

Figure 2 Effect of ethanolic extract (EZHm) on inflammation caused by CFA

Compared to the hematocrit level of untreated rats that of treated rats increased insignificantly ($p > 0.05$). Analysis of the results shows a non-significant drop ($p > 0.05$) between the number of blood platelets in the untreated rats and that of the rats treated with Diclofenac and DZHm at doses of 200 and 400 mg/kg bw. However, a significant reduction ($P<0.05$) in this parameter was observed with the rats treated with DZHm at the dose of 800 mg/kg bw (Table 1).

Regarding the rats treated with the hydro-ethanolic extract of the ZHm recipe, no significant difference ($p > 0.05$) was observed in the number of red blood cells, the hemoglobin level, the hematocrit and the number of blood platelets. However, fibrinogen levels decreased significantly ($p<0.01$), ($p<0.0001$) and ($p<0.001$) in rats treated with doses of 200, 400 and 800 mg/kg bw of extracting respectively and compared to that of untreated rats. These values were 2.7 g/L; 1.75 g/L and 2.07 g/L for rats treated respectively with EZHm at doses of 200, 400 and 800 mg/kg bw. At a dose of 800 mg/kg bw, this extract very significantly lowered ($p<0.001$) the number of white blood cells in rats (11.17±0.62) (Table 2).

Table 1 Effect of different doses of DZHm and Diclofenac on hematological parameters of arthritic rats after 20 days of treatment

Parameters	Healthy contols	Untreated contols	Diclofenac 5 mg/kg/bw	200 mg/Kg/ bw	400 mg/Kg/ bw	800 mg/Kg/ bw
White blood cells 10^3/µL	15.58± 2.05	21.12± 0.68	12.45 ± 1.68^b	14.01 ± 1.59^a	12.76 ± 0.66^b	12.12 ± 2.54^b
Red blood cells 10^6/µL	7.59±0.12	6.67 ± 0.4	7.67±0.37^a	6.78±0.21	8.28±0.16^b	8.10±0.3^a
Hemoglobin g/dL	12.6±1.02	12.03±1.01	12.63±0.44	12.75±0.35	13.88±0.41^a	14.13±0.22^a
Hematocrit (%)	4418±4.8	39.9±3.7	46.8±2.02	43.1±1.33	45.68±0.52	47.38±1.71
Platelets	774.5±24.5	988.8±11.4	850 ±11.42	913,3±21.2	730.5 ± 26.7^a	774.1 ± 24.81
Fibrinogen (g/L)	2±0.2	3.5±0.14	2±0.7^b	2.3±0.07^b	1.62±0.16^d	1.65±0.12^d

Table 2 Effect of different doses of EZHm and Diclofenac on hematological parameters of arthritic rats after 20 days of treatment

Parameters	Healthy contols	Untreated contols	Diclofenac 5 mg/kg/bw	200 mg/Kg/ bw	400 mg/Kg/ bw	800 mg/Kg/ bw
White blood cells 10^3/µL	15.58± 2.05	21.12± 0.68	12.45 ± 1.68^a	16.08 ± 3.66	15.34±1.55	11.17±0.62^C
Red blood cells 10^6/µL	7.59±0.12	6.67 ± 0.4	7.67±0.37	7.10±0.6	8.01±0.22	7.76±0.25
Hemoglobin g/dL	12.6±1.02	12.03±1.01	12.63±0.44	12.48±0.19	13.15±0.23	13.23±0.3
Hematocrit (%)	44.18±4.8	39.9±3.7	46.8±2.02	41.05±1.3	44.25±1.39	44.35±1.42
Platelets	774.5±24.51	988.8±11.4	850 ±11.42	933.8±25.2	856.5±18.8	845.8±16.7^a
Fibrinogène (g/L)	2±0.2	3.5±0.14	2±0.7^b	2.47±0.07^b	1.75±0.12^d	2.07±0.3^c

Data were expressed as mean ± SEM. Significant differences were expressed between untreated rats and rats treated with the different substances.
-not significant (ns): $p > 0.05$;
- Insignificant (a): $p < 0.05$;
- Significant (b): $p < 0.01$;
- Highly significant (c): $p < 0.001$;
- Highly significant (d): $p < 0.0001$
DZHm / EZHm : decocted / Hydroethanolic extract of the mixture of *Z. leprieurii, H. madagascariensis and X. aethiopica*

4. Discussion

Dysregulation or failure to resolve acute inflammatory responses leads to chronic inflammation that results in necrosis and fibrosis [**12**]. The model for studying chronic inflammation is adjuvant-induced arthritis in rats. Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterized by inflamed synovial membrane, cartilage destruction, altered joint integrity, polyarthritis with functional impairment and disability. Although the exact cause remains unclear, pro-inflammatory mechanisms involving cytokines, particularly tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL- 1β), have been implicated by several studies as being associated with disease progression and joint destruction [**2,3**]. In an attempt to prevent or mitigate inflammation in sickle cell disease, chronic inflammation was induced in rats by injection of Freund's Complete Adjuvent (FCA) to verify the curative effect of ZHm extracts.

Injection of CFA induced local inflammation that c developed into ulceration, resulting from the action of several chemical mediators, such as prostaglandins, histamine, and serotonin according to Soliman and Barreda [12]. These findings are consistent with Stills [13], who showed that immune modulation by CFA results in inflammation, tissue

destruction, and risk of pain and distress in the host animal. Adjuvant-induced arthritis in the host animal develops in two phases : a phase of acute periarticular inflammation followed by a phase of chronic inflammation with joint and bone involvement [11, 14].

One of the characteristics of chronic inflammation is the loss of body weight. This weight loss is a phenomenon well illustrated in Fig 1-A and Fig 2-A, in which a decrease in weight is observed on day 2e of the study. Overall, all curves show a progressive upward profile revealing a gradual recovery of weight. This is consistent with an action of the rats' immune system. Detailed observation of the results reveals that compared to untreated animals, weight gain occurs rapidly in all animals treated with 800 mg/kg bw ZHm, which was more effective than all substances tested. The loss of body mass involves a clear catabolic process, with negative energy, protein and micronutrient balances over a period of time. The presence of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α have been reported to mediate body mass loss that results from chronic inflammation [15]. This is in agreement with the observation made in this study where arthritic rats had significantly lower body weight than non-arthritic rats. Treatment of rats with the different extracts of the ZHm recipe reportedly led to inhibition of the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α. According to Masengo [3], these pro-inflammatory cytokines have been reported to mediate the loss of body mass that results from chronic inflammation.

In general, in the arthritic control, the analysis of the results shows that the volume of edema increases rapidly until it peaks at day 4e and then continues slowly until day 20^{e} compared to the untreated rats. All other treated batches had slight edema at 4e day which gradually decreases in volume, until 21e day. The volume of the treated paws was smaller than at 1e day of observation. Thus, these substances were successful in reducing the edema. The 800 mg/kg bw dose of ZHm has similar activity to Diclofenac. This capacity, to reduce the oedemas by the extracts would be related to the presence of the phenolic compound. Indeed, the polyphenols are recognized for their activities on the cutaneous lesions to boost the immune system.

Daily administration of the ZHm extracts caused a dose-dependent reduction in edema. The anti-inflammatory effect of ZHm extracts could be explained by the antagonistic substances in the biosynthesis of these chemical mediators. According to studies conducted by Koua [16]**;** polyphenols and their derivatives would inhibit the enzymatic activity of arachidonic acid and reduce the production of inflammation mediators such as arachidonic acid, nitric oxide, prostaglandins and leukotrienes [17,18]**.**

In addition to phenolic compound, triterpenoids are known to exert anti-inflammatory effects via activation of Nrf2, leading to transcription of enzymes including HO-1 [19]. Phenolic compound, sterols, terpenes, and saponins present in the ZHm recipe extracts are thought to inhibit inflammatory cell migration. In addition, the antioxidant properties of phenolic compound, sterols, terpenes could reduce free radicals produced during inflammation [20]. The anti-inflammatory effect of ZHm extracts could be explained by substances antagonistic to the biosynthesis of these chemical mediators. According to studies conducted by Koua [16] and Abizi [15]; polyphenols and their derivatives would inhibit the enzymatic activity of arachidonic acid and reduce the production of inflammation mediators such as arachidonic acid, nitric oxide, prostaglandins and leukotrienes [17 ; 21]. In addition to phenolic compound, triterpenoids are known to exert anti-inflammatory effects via activation of Nrf2, leading to transcription of enzymes including HO-1 [19]. The phenolic compound, sterols, terpenes and saponins present in ZHm recipe extracts are thought to inhibit inflammatory cell migration. In addition, the antioxidant properties of phenolics, sterols and terpenes could reduce the free radicals produced during inflammation [20]. The work of Tatiana [22] reveals the presence of these compound in this plant recipe. Physiologically, these compound would participate in the activation of the classical complement pathway, mobilization and activation of leukocytes, stimulation of phagocytosis and secretion of cytokines by monocytes [23].

Anemia of chronic disorders is characterized by a reduction in iron levels, red blood cell survival, iron-binding capacity, and the inability of the bone marrow to increase erythrocyte production. This is usually associated with chronic infection, inflammation and malignancy [24]. The progression of inflammation is associated with a reduction in red blood cell count, hemoglobin and hematocrit [25]**.** These extracts have been shown to mitigate inflammation and associated anemia. This was evidenced by a reduction in the number of white blood cells, fibrinogen levels and blood platelets and a significant increase in the number of red blood cells, hemoglobin and hematocrit levels compared to untreated rats. These extracts, by reducing the fibrinogen level and the platelet count in rats treated with the extracts and Diclofenac, could participate in the reduction of blood viscosity in sickle cell subjects. The effect of the extracts on the key parameters of anemia and the decrease of WBC, PLT and fibrinogen level would mean that these extracts corrected the anemia and the infection caused by CFA, this effect could thin the blood of sickle cell patients.

These observed effects on blood parameters indicate the potential of ZHm extracts to inhibit the progression of inflammation. The anti-inflammatory activity of the aqueous and hydroethanolic extracts of the ZHm recipe is thought

to be due to the synergistic effect of the chemical compounds present in *Z. leprieurii, H. madagascariensis and X. aethiopica.* According to Obiri [26], Medewase and Ezike [27], Zondegoumba [28] and Evelyne [29], the presence of saponins, phenolic compounds, alkaloids and essential oils in the crude extracts from the fruits of *Xylopia aethiopica;* the leaves of *H. madagascariensis* and the barks of *Z. leprieurii* inhibit the oedemas produced by the CFA and Carrageenan These authors also showed that these extracts had an antioxidant power linked to the presence of these molecules. The extracts of ZHm could be considered as anti-inflammatory products.

Definitions, Acronyms, Abbreviations

- DZHm: Decocted extract of *Zanthoxylum leprieurii, Xylopia aethiopica,* and *Harungara madagascariensis* combination.
- EZHm: ethanolic extract of *Zanthoxylum leprieurii, Xylopia aethiopica,* and *Harungara madagascariensis* combination.

5. Conclusion

In view of the inflammatory context present in sickle cell disease, the question of a therapeutic development based on the administration of anti-inflammatory agents naturally arose. The use of this traditional herbal formulation inhibited the progression of inflammation induced by CFA, the proliferation of leukocytes, the fibrinogen level and also increased the hemoglobin level which is the key parameter of anemia. These observed effects are due to the antioxidant power of phenolic compound and alkaloids, terpenoids present in ZHm extracts.The therapeutic virtues of the ZHm recipe justify its use against sickle cell disease in the traditional environment of Indénié-Djuablin.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The investigation conforms to the recommandation of OECD in 2008.

Statement of informed consent

An agreement was obtained from the ethic committee and an informed consent approved by each patient wishing to participate to the study.

Competing interests

Authors have declared that no competing interests exist.

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